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CHAPTER

7

Nanoparticle-based vaccines: opportunities and limitations

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1. Chapter outline

1.1 Introduction

Nanomedicine has seen increased interest by the medical community in recent years, as it has enabled modification of engineering devices for delivery to and interaction with cell environments. In particular, this technology has enabled advances in the design of delivery systems for drugs and vaccines [1]. Nanoparticles (NPs) are now being engineered from different biodegradable materials, including natural and synthetic polymers (poly[lactic-co-glycolic] acid (PLGA) and polylactic acid (PLA)), metals (gold, copper oxide, aluminum oxide, zinc oxide, iron oxide, and silver), or lipids (phosphatidylserine, phosphatidylcholine, and cholesterol) [2].

NPs and microparticles (MPs) have been widely used to deliver drugs, especially cytotoxic drugs or immune-suppression treatments for transplantation. These NPs are effective in the distribution of medications to target specific organs and control drug delivery. These drug carriers not only transport drugs to sites of cancer or other target diseased organs, preventing damage to healthy cells, but also protect the drug from degradation [3]. Some of the metal nanoparticles (e.g., gold, silver, or PLGA) themselves may have dual functions, working both as carriers and as targeted delivery systems using the membranes of cancer cells, which contain tumor-associated markers. In a recent study, Fang et al. used a membrane from mouse melanoma cancer cells in the outer layer of PLGA nanoparticles. These NPs were stable and persisted in the structure during cellular endocytosis, activating the maturation of dendritic cells (DCs) and presenting especially to T cells that have TCR binding to gp100 and inducing the production of IFN-γ. Moreover, the authors found that the PLGA covering membrane had receptors that allow interaction with

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cancer cells and delivery of the drug [4,5]. Furthermore, the NPs were used to deliver drugs to the specific site of organ transplantation to prevent rejection. Systemic immunosuppression is a high risk for recipients of organ transplantation, as it uses high doses of immunosuppression agents that make them more susceptible to infections and in some cases cause death. Previous studies found that diabetic mice were transplanted with islets into the eye to prevent islet graft immune rejection in vivo using rapamycin MPs. The islets transplanted with the immunosuppressed drug MPs survived more than 1 month compared with the control (empty MPs), which rejected the islets in the second week [6].

Work in last few decades has increased our knowledge of infectious diseases and the mechanisms of evasion of immune responses. However, new variants of antibiotic-resistant pathogenic microorganisms have emerged and are becoming a challenge for designing new vaccines and adjuvants. Until now vaccines have been developed from live-attenuated microorganisms or killed pathogens (first-generation vaccines) [7], DNA vaccines (third-generation vaccines), subunit vaccines [8], and synthetic peptides (second-generation vaccines). The last three vaccine types eliminate the risk of developing the disease, but they must be used in conjunction with an adequate adjuvant or delivery system (Table 7.1). The combination of the vaccine with the adjuvant or delivery system should be safe, stable, and have the ability to induce long-lived memory B and T cell responses, preferably with a single dose and a maximum of two doses and be free from strict storage requirements [9]. DNA and RNA vaccines are safe but need a second boost with recombinant protein or DNA from another vector. NPs have become an alternative to targeting vaccine delivery to immune cells, improving vaccine efficacy with slow release, easy antigen uptake, and induction of humoral and cellular responses [8].

NPs have played an important role in the activation of antigen-presenting cells (APCs), especially DCs, which may determine vaccine efficacy. Although there is some cytotoxic effect of the NPs [10,11], the risk is low compared with the benefits of vaccine delivery [12]. In this chapter, we will summarize the different nanocarrier-based vaccine formulations that achieve the desired host immunity against infectious diseases and cancer, and at the end, we will discuss on the limitations of the respective carriers.

1.2 Immune responses after vaccination

DCs are specialized APCs that coordinate the innate and adaptive immune responses to

TABLE 7.1 Types of vaccines.

Vaccine	Constituent	Examples
Live-attenuated vaccines	Whole pathogen	Measles, mumps, rubella, rotavirus, smallpox, chickenpox, yellow fever
Inactivated vaccines	Whole pathogen	Rabies, flu, polio, hepatitis A
Subunit, recombinant, polysaccharide, and conjugate vaccines	Part of the pathogen	Hepatitis B, whooping cough, Hib (*Haemophilus influenzae* type b) disease, human papillomavirus, shingles, meningococcal disease, pneumococcal disease
Toxoid vaccines	Toxin	Diphtheria, tetanus
Future of vaccines	DNA	Research studies

induce "talking" by chemokines to start the defense against infectious diseases. There are three subtypes: plasmacytoid, myeloid, and follicular DCs. Depending on the subtype of DC, the cytokine profile will be different, and the responses may be protective or not. The interaction and delivery of antigens and adjuvants to DCs is a research priority, in order to optimize the humoral and cellular vaccine responses [13]. Follicular DCs are the key to activating naïve T and B cells to initiate the adaptive immune response and the induction of long-lived memory cells. Lymphocyte activation is started after recognition of the antigen by the T cell receptor (TCR) and B cell receptor (BCR). The recognition of a specific antigen is associated with reorganization of costimulatory surface proteins such as CD40 ligand, CD28, CD4, or CD8 in T cells and CD40, CD80, and CD86 in APCs. The early molecular events that underlie the formation of the synapse are highly coordinated and tightly controlled. The B cells spread over the antigen- APC- C3 or FcR or CR, rapidly, preventing antigen internalization and phagocytosis by DCs and their posterior presentation to MHC class I or class II. Before the antigen is processed and presented along with MHC class II molecules to the TCR, antigen−MHC complexes mediate the recruitment of naïve $CD4^+$ T helper cells. The DCs present the antigen−MHC class II complex to the TCR, and the T cells differentiate into one of the subtypes of $CD4^+$ T helper cells (Th). The interaction of B cell MHC class II−antigen complexes with the TCR of $CD4^+$ T cells is necessary for full B cell activation and production of antibodies [14]. The DCs are the principal target for delivery of the vaccine and hence for NP, although other APCs, such as macrophages, B cells, and lung epithelial cells, can present the antigen to T cells. Unlike DCs, macrophages induce high lysosomal activity after phagocytosis to enhance antigen presentation and thus effector immune responses. Nanotechnology has been improving antigen delivery, depending on the size, charge, and type of NP that can enter through receptors, such as Toll-like receptors (TLRs), pinocytosis, phagocytosis, or special receptor targets. The entry of DNA vaccines using different types of NPs is shown in Fig. 7.1 [5].

1.3 Types of NPs used to deliver vaccines

Until now there have been various types of NPs, composed of gold, dendrimers, carbon, polymers, and liposomes, that have been used to deliver vaccines. All can stimulate the production of cytokines and antibody responses [15−19]. The cargo is not limited to vaccines, and it is possible to add adjuvants and immune stimulatory molecules, including silica and iron, TLR agonist, and cytokines, to improve immunogenicity [20,21]. Several vaccines have been tested on the different types of NPs (Table 7.2).

1.4 Liposomes

Liposomes are the second-most common type of NP, and they self-assemble in water under special conditions. They are composed of lipids, which have a hydrophilic head and a hydrophobic tail that maintain hydrophilic inner and outer membranes, in lamellar lipid bilayers or in multilayers that simulate vesicles found within cells [22]. Liposomes can induce cellular responses or humoral responses, depending on the charge, size, and lipid composition. A previous study showed that charge had the main role in activation of the cellular or humoral immune responses. The authors' approach was to investigate the importance of the antigen−liposome interaction in immunogenicity and depot formation. They used subunit antigens Ag85B−ESAT-6 (pI = 4.9) from TB, and "CTH1" (pI = 9.0), from *Chlamydia* vaccines and a model antigen, lysozyme (pI = 11). The injection of cationic dimethyldioctadecylammonium

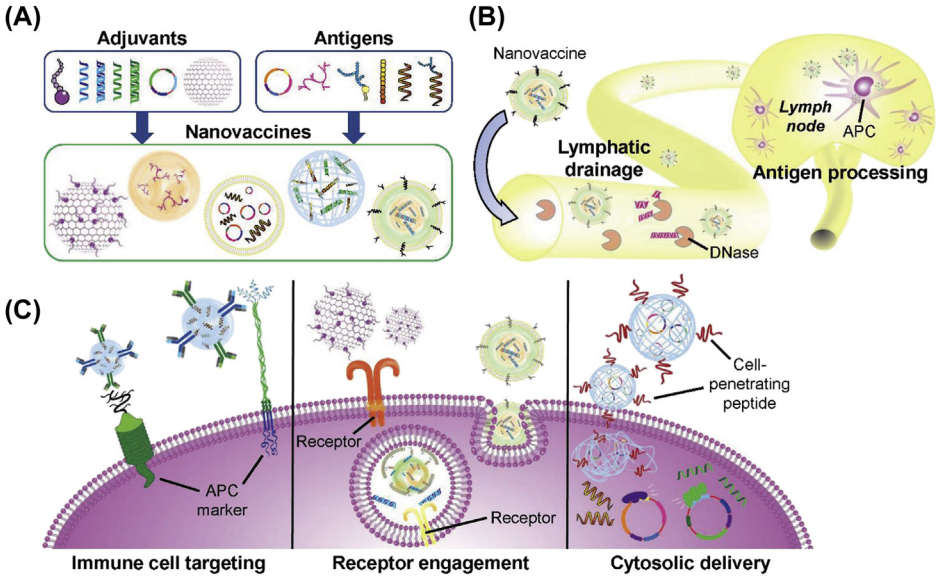

FIGURE 7.1 Advantages of NPs for vaccine design. (A) Various combinations of adjuvants and antigens can be formulated using NP platforms such as liposomes, emulsions, nanogels, and other substances. (B) Nanovaccines can access the lymphatic drainage system for lymph node delivery while protecting their cargoes from environmental degradation. Once at the lymph nodes, the nanocarriers deliver their cargoes to APCs for immune processing. (C) Nanovaccine properties can be tuned to efficiently deliver their cargoes for maximum immune activation. For example, NPs can be modified to target specific subsets of immune cells. They can also be delivered to specific intracellular compartments, where receptors for immune pathways can be triggered. *From Kroll AV, Jiang Y, Zhou J, Holay M, Fang RH, Zhang L. Biomimetic nanoparticle vaccines for cancer therapy. Adv Biosyst 2019:1800219 with permission.*

TABLE 7.2 List of antigens delivered by nanocarriers for the treatment of different diseases in vaccine research.

Antigen	Nanocarrier used	Disease
Against Bacterial Infection		
Antigenic protein	Poly(D,L-lactic-co-glycolic acid) nanospheres	Anthrax
DNA encoding T cell epitopes of Esat-6 and FL	Chitosan nanoparticle	Tuberculosis
Mycobacterium lipids	Chitosan nanoparticle	Tuberculosis
Polysaccharides	Liposomes	Pneumonia
Bacterial toxic and parasitic protein	Liposomes	Cholera and malaria
Fusion protein	Liposomes	*Helicobacter pylori* infection
Antigenic protein	Nanoemulsion	Cystic fibrosis
Antigenic protein	Nanoemulsion	Anthrax
Mycobacterium fusion protein	Liposome	Tuberculosis

TABLE 7.2 List of antigens delivered by nanocarriers for the treatment of different diseases in vaccine research.—cont'd

Antigen	Nanocarrier used	Disease
Against Viral Infection		
Antigenic protein	Chitosan nanoparticles	Hepatitis B
Viral protein	Gold nanoparticles	Foot and mouth disease
Membrane protein	Gold nanoparticles	Influenza
Viral plasmid DNA	Gold nanoparticles	HIV
Tetanus toxoid	Poly(D,L-lactic-co-glycolic acid) nanospheres	Tetanus
Hepatitis B surface antigen	Poly(D,L-lactic-co-glycolic acid) nanospheres	Hepatitis B
Hepatitis B surface antigen	Alginate-coated chitosan nanoparticle	Hepatitis B
Live virus vaccine	Chitosan nanoparticles	Newcastle disease
Capsid protein	VLPs	Norwalk virus infection
Capsid protein	VLPs	Norwalk virus infection
Influenza virus structural protein	VLPs	Influenza
Nucleocapsid protein	VLPs	Hepatitis
Fusion protein	VLPs	Human papilloma virus
Multiple proteins	VLPs	Rotavirus
Virus proteins	VLPs	Blue tongue virus
Enveloped single protein	VLPs	HIV
Viral protein	Polypeptide nanoparticles	Corona virus for severe acute respiratory syndrome (SARS)
Against Parasitic Infection		
Merozoite surface protein	Iron oxide nanoparticles	Malaria
Epitope of *Plasmodium berghei* circumsporozoite protein.	Polypeptide nanoparticles	Rodent malarial parasitic infection
Surface protein from *Eimeria falciformis* sporozoites	ISCOMs	Diarrhea

From Pati R, Shevtsov M, Sonawane A. Nanoparticle vaccines against infectious diseases. Front Immunol 2018;9. adapted with permission.

bromide (DDA): trehalose 6,6′-dibehenate (TDB) liposomes with associated Ag85B—ESAT-6 form a deposit at the site of injection. By contrast, the anion disappears immediately. The cationic liposomes induce the infiltration of monocytes at the site of immunization, possibly mediated by depot formation. These liposomes induce strong T cell proliferation and differentiation through Th1 and Th17 responses, although the authors could not find DCs. However, DCs are known

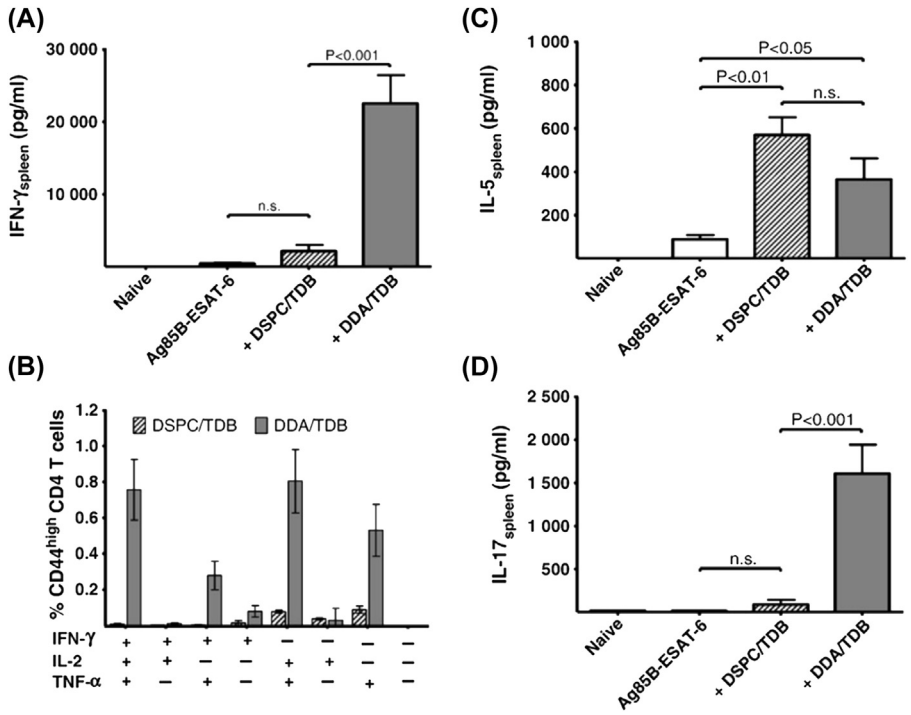

FIGURE 7.2 Immune responses in mice 3 weeks after the last of three immunizations with 2 μg of Ag85B–ESAT-6 alone (white) or in combination with DSPC–TDB (dashed) or DDA–TDB (gray). (A) IFN-γ responses in the spleen. (B) Frequency of $CD44^{high}$ T cells in response to each of eight possible cytokine subsets of IFN-γ, IL-2, and/or TNF-α. (C) IL-5 responses in the spleen. (D) IL-17 responses in the spleen. *Adapted from Henriksen-Lacey M, Christensen D, Bramwell VW, Lindenstrøm T, Agger EM, Andersen P, et al. Liposomal cationic charge and antigen adsorption are important properties for the efficient deposition of antigen at the injection site and ability of the vaccine to induce a CMI response. J Control Release 2010;145(2):102–8 with permission.*

to migrate to the secondary lymph nodes to activate the T and B cells, which the authors did not analyze in this manuscript [23]. Previous studies have shown that DDA induces proinflammatory cytokines and chemokines after infiltration of monocytes and macrophages in vivo [24]. One of the advantages of liposomes is mimicking properties of the pathogens, inducing humoral and cellular immune responses. The antigen's presentation to APCs depends on the membrane characteristics, size, and ligand-receptor binding. Liposomes could induce Th2 responses if the lipids are unsaturated whereas saturated lipids promoted Th1-type immune responses [25,26]. Liposomes could be modified according to the needs of delivery to produce Th1, Th2, Th17, or Tfh immune responses. Cationic liposomes can increase the uptake of subunit vaccines as synthetic peptides and recombinant proteins; this can be explained by the interaction between positively charged liposome with the APCs that have a negative charge in their membrane [26,27]. Another modification is the pH-sensitive fusogenic liposomes, which are stable in neutral pH 7.4, but in acidic conditions, the antigen is released and presented by MHC class I or II. These liposomes containing phosphatidylethanolamine and amphiphilic stabilizers allow PE-containing liposomes to form aggregates, due to the poor hydration of their headgroups, which can explain their high affinity to adhere to cell membranes [28,29].

Another study designed a unique structure composed of inter-bilayer-crosslinked multilamellar vesicles (ICMVs), which is stable in the extracellular environment but rapidly released in endosomes/lysosomes, thereby enhancing vaccine immune responses. The ICMVs carried the antigen OVA mixed with the adjuvant monophosphoryl lipid A (MPLA). This mixture amplified vaccine responses and upregulated the costimulatory cells on splenic and bone marrow DCs. In addition, splenic DCs incubated with the OVA^+ vaccine MPLA triggered proliferation of naïve OT-1 $CD8^+$ T cells in vitro, suggesting that the ICMVs enhanced cross-presentation of the antigen. The same results were obtained in vivo after the vaccination of mice with the mixture, and the ICMVs elicited robust antibody titers that were ~1000-fold greater than simple liposomes. These results could be attributed to activation enhancement of DCs and antigen cross presentation [30]. The first use of liposomes as a delivery system for a malaria vaccine was in the 1980s. Ballou et al. synthetized peptides derived from the repetitive region of the circumsporozoite (CS) protein of *Plasmodium falciparum* sporozoites. The synthetic peptides were conjugated to keyhole limpet hemocyanin (KLH) proteins and were incorporated into liposomes. Immunized mice and rabbits produced antibodies against the repeat region of the protein with biologic activity correlated with protection [31]. RTS, S is the only vaccine against malaria that is in phase three clinical trials in Africa. The RTS, S vaccine has the central repeat region of CSP, and T cell epitopes localized in the C-terminal region are fused to hepatitis B surface antigen (HBsAg) and expressed in *Saccharomyces cerevisiae* yeast. This virus-like particle (VLP) vaccine also contains MPLA and QS-21, which enhances humoral and $CD4^+$ T cell responses in the first 4 years, with a level of protection of 18%–36%. Protection decreased rapidly thereafter, with negative efficacy in some children. The problem is not RTS, S nor the adjuvant system, as both induce potent immune responses. However, the CSP part of RTS, S is an antigenic polymorphism, and T cell epitopes present on the CS protein that are incorporated into the vaccine are also polymorphic. To address this problem, the newest generation of this vaccine is coformulated with Matrix-M, a saponin-based liposomal adjuvant [32]. However, the vaccine protection against *P. falciparum* has not been achieved due to the short-lived memory cells and the evasion mechanisms of the parasite [33]. The first malaria vaccine was tested in 1967 in animals using *Plasmodium bergheiś* radiation-attenuated sporozoites, with a 100% of protection [34]. Hoffman et al. reported in 2002 that 10 human volunteers were vaccinated with irradiated sporozoites of *P. falciparum* strain NF54, and all of them were protected between 2 to 9 weeks [35]. This vaccine will be used to vaccinate 360,000 children a year in three African countries (Ghana, Malawi, and Kenya). The vaccine could prevent 4 in 10 cases according to previous clinical trials.

1.5 Virus-like particles

Virus-like particles (VLPs) are composed of a self-assembling viral membrane maintaining viral surface proteins. VLPs can be modified to express additional proteins of other microorganisms, which could be engineered by fusion of the proteins with membrane antigens or by endogenous expression of other antigens [36]. Gardasil is a four-component VLP-type vaccine specific against HPV. It contains the L1 major capsid protein of HPV types 6, 11, 16, and 18 and is administered along with an aluminum adjuvant. This vaccine was administered to 1158 women and was followed up for incidence of persistent associated infection for 35 months. The efficacy was 100% for preventing clinical disease. The HPV combination vaccine was immunogenic, inducing the production of long-lived antibodies [37]. However, some problems with the vaccine in teenage girls were reported.

The adverse events included dermatologic/mucosa-allergic reaction (25%), rash (22%), and local/injection-site reaction (20%). However, some serious adverse events following immunization were reported (7.5% of reports), including two incidents of anaphylaxis, two seizures, one incident of thrombocytopenia, and one death [38].

1.6 Metal and nonmetal inorganic NPs

Inorganic metal NPs are frequently used in drug delivery and bioimaging, especially in treating cancer patients. DNA vaccines are more stable and protected from degradation when carried by a gold, silica, or silver NP delivery system. The covalent attachment of Chito6 to GNPs increases the NP molecular weight, enhancing DNA binding and stability without compromising DNA release and transfer. Chito6−GNP−DNA (HBsAg) complexes induce effective antibody and T cell responses after immunization of BALB/c mice. By contrast, naked DNA-primed HBsAg induces antibodies after a series of four immunizations with 10 mg of naked DNA. HBsAg-specific $CD8^+$ T cells eliminated P815/BALB target cells that had been sensitized with an HBsAg CTL epitope peptide in vitro. These chimeric NPs, employing a minimal amount of DNA, induce effective immune responses when compared with naked DNA [39].

1.7 Polymeric NPs

Biodegradable polymers are of significant interest in the delivery of drugs and vaccines against infectious diseases. These polymers consist of either natural or synthetic monomers that are biodegradable, are nonimmunogenic, have low cytotoxicity, and are easy to prepare. There are several polymers, such as chitosan, PLGA, polyethylene glycol (PEG), polycaprolactone, and dextran used as delivery systems [40]. PLGA is approved for human use by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), while PLGA has been used to deliver drugs for long periods. DCs play an important role in the activation of adaptive immune responses, in which B cells and T cells activate and differentiate into subpopulations that determine which humoral or cellular immune responses eliminate the microorganism. Cruz et al. studied the delivery of NPs and MPs to DCs to create a biocompatible and biodegradable slow-release vaccine and effectively target the cells. The NPs and MPs (PLGA−PEG) were loaded with tetanus toxoid peptide−FITC linked to a Lys−Lys cathepsin cleavage site and an anti-DC-SIGN antibody. The DCs targeted PLGA-based vaccine NPs but not MPs. The NPs were efficiently taken up with the help of anti-DC-SIGN antibodies and induced proliferation of T cells (Fig. 7.3). However, the MPs were taken up for all APCs, including the DCs (Fig. 7.4). New research is needed to understand the biology, antigen processing, and presentation needed to induce long-lived memory B and T cells [41].

Botulism is a lethal neuroparalytic disease produced by *Clostridium botulinum* toxins (A-H). Ruwona et al. demonstrated that cationic PLGA NPs can carry plasmid DNA encoding the BoNT heavy-chain (Hc) fragment and that its product is nontoxic. Immunized mice produced high titers of antibodies after 5 to 9 weeks (Fig. 7.5).

After four immunizations, specific IgG1 had decreased, but IgG2a had increased, compared with the first immunization. After challenge, 100% of the mice vaccinated with PLGA−pVax/opt-BoNT/C-Hc50 survived, while only 80% of the mice immunized with naked plasmid were protected (Fig. 7.6) [17].

Modifications of polymeric NPs have been used to deliver synthetic peptides or recombinant proteins to dendritic cells and macrophages.

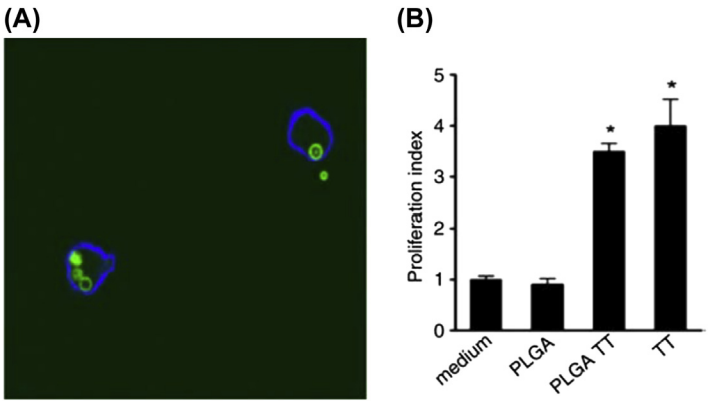

FIGURE 7.3 Uptake of PLGA MPs with FITC−TT peptide by DCs results in antigen presentation. DCs were incubated with FITC−TT containing MPs (green; light gray in printed version) for 1 h to confirm uptake by human DCs. Cells were analyzed by confocal laser scanning microscopy. The cell surface was visualized by MHC class II staining (blue; white in print version). The image represents the middle focal plane of the DCs, with the iris set at 2 nm (A). Presentation of PLGA-encapsulated FITC−TT peptide was studied by culturing DCs for 18 h in culture medium alone, medium supplemented with empty PLGA MPs (PLGA), or with 0.1 μg of FITC−TT peptide encapsulated within PLGA MPs (PLGA TT). In addition, DCs were pulsed with 1 μM TT peptide as a positive control for antigen presentation (TT). Subsequently, autologous TT-responsive peripheral blood lymphocytes were added. After 3 days, cellular responses were assessed in a proliferation assay. Data are mean proliferation indices ± SD relative to medium control for experiments performed in triplicate. Significant differences from medium control according to ANOVA and Dunnett's test:*$P < .01$. *From Cruz LJ, Tacken PJ, Fokkink R, Joosten B, Stuart MC, Albericio F, et al. Targeted PLGA nano-but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN in vitro. J Control Release 2010;144(2):118−26 with permission.*

Salvador et al., modified PLGA MS, 50:50 lactide−glicolide ratio, to produce cationic NPs using polyethylene imine (PEI), and BSA as antigen. In addition, the NPs were modified encapsulating monophosphoryl lipid A (MPLA) or polyinosinic-polycytidilic acid poly(I:C) and α-galactosyl ceramide to address their adjuvant effect. Coumarin 6 was incorporated into the oily phase for the preparation of fluorescent NPs to evaluate in vitro assays. The NPs were tested in vitro using monocytes and dendritic cells, and mice were immunized with the different NP modifications to determine the immune responses in vivo. The cationic NPs showed a noticeable enhancement of their uptake by the monocytes, MDCs, and PDCs in comparison to the classical NPs. In addition, the cationic NPs increased immunostimulatory effect since they induced the production of antibodies and Th1 cell responses producing IFN-γ [12].

1.8 NP-investing companies and clinical trials

The interest of companies in nanoparticle-based vaccine delivery dramatically increased in the last decades. Pevion Biotech Ltd., a Swiss company founded in 2002, developed the virosome-based technology platforms to make efficient and safe prophylactic/therapeutic vaccine candidates. For *Candida albicans* that can cause vulvovaginal mucosal infections, Pevion used the aspartyl-proteinase (Sap2), which is an immunodominant antigen and virulence factor; this recombinant protein was assembled with virosomes (PEV7). The evaluation in mouse model and the initial clinical trial on women showed that this candidate vaccine, intravaginally administered, has a therapeutic potential for the treatment of recurrent candidiasis [42]. In addition, this company tested a malaria vaccine in Africa. This vaccine had

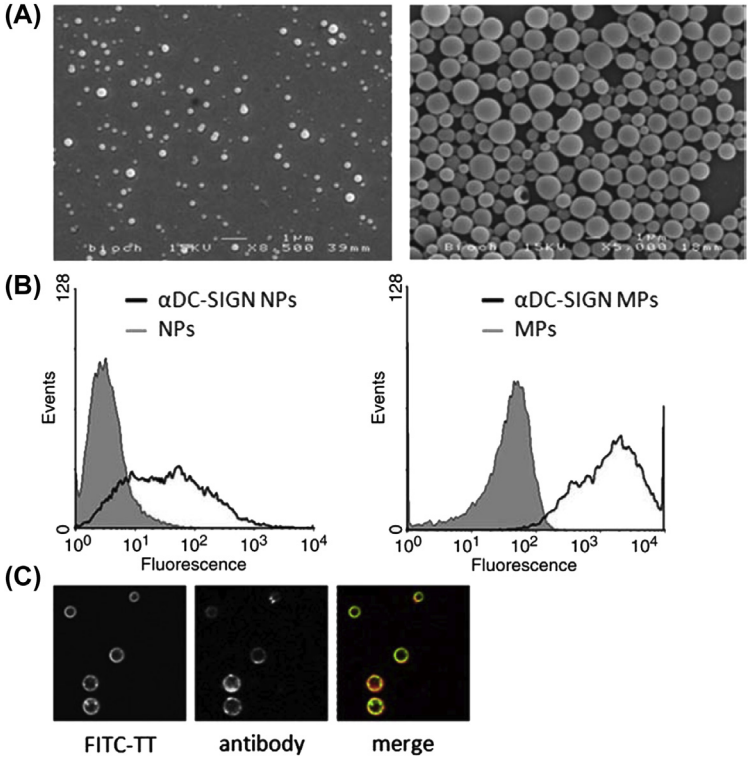

FIGURE 7.4 Antibodies were introduced on the surface of PLGA NPs and MPs. The morphology of NPs (A, left panel) and MPs (A, right panel) with PEG–lipids was analyzed by scanning electron microscopy. The presence of the antibodies on the PLGA particle surface was confirmed by flow cytometry. The NPs and MPs were stained with fluorescence-labeled secondary antibodies and analyzed on a flow cytometer (B). PLGA MPs were mounted on glass slides and analyzed by confocal laser scanning microscopy to visualize antibodies present on the particle surface. FITC–TT peptide was detected as a green (light gray in print version) *fluorescent ring* surrounding the PLGA particles (see also Fig. 7.2A). Antibodies on the particle surface were detected by secondary antibody staining with Alexa 647-labeled anti-human IgG. The images represent the middle focal plane of particles and show split channels of the FITC signal (FITC–TT), the Alexa 647 signal (antibody), and a merged image showing the antibody in red (dark gray in print version) and the FITC–TT peptide in green (white in print version) (C). *From Cruz LJ, Tacken PJ, Fokkink R, Joosten B, Stuart MC, Albericio F, et al. Targeted PLGA nano-but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN in vitro. J Control Release 2010;144(2):118–26 with permission.*

combination of FFM ME-TRAP+PEV3A (AMA-1) and was tested in phase I/IIa clinical trials. Although the vaccine did not show sterile protection, it induced responses on blood stage parasites and lower rates of parasite growth in human volunteers vaccinated with PEV3A, compared to unvaccinated controls [43]. GlaxoSmithKline plc is working to improve the coformulated RTS, S vaccine using Matrix-M, a saponin-based liposomal adjuvant [32]. Novavax is developing respiratory syncytial virus (RSV) F nanoparticle vaccine with aluminum. The purpose of the reported study was to evaluate the safety and efficacy of maternally transferred antibodies in preventing RSV in infants. The vaccinated healthy third-trimester pregnant women showed significant protection to newborn children from RSV challenge and reduced pulmonary inflammation, and the vaccine was safe and effective for maternal and

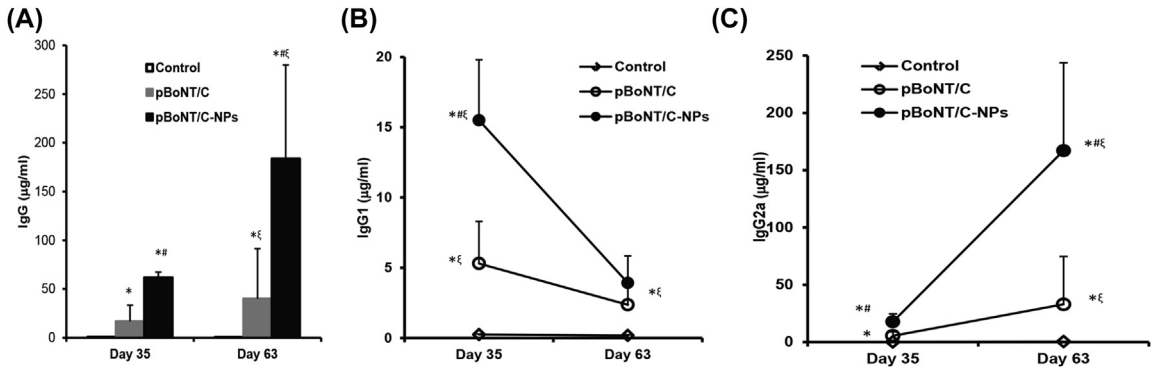

FIGURE 7.5 Serum anti-BoNT/C-Hc50 IgG (A), IgG1 (B), and IgG2a (C) induced by intramuscular immunization of mice with pVax/opt-BoNT/C-Hc50 (20 μg/mouse), alone (pBoNT/C) or coated on cationic PLGA NPs (pBoNT/C-NP). SKH-1 Elite mice (n = 5) were dosed in weeks 0, 2, 4, and 8. Control mice received PBS only. Blood samples were collected in week 5 (day 35) and week 9 (day 63). Data are mean ± SD (n = 5). *$P < .05$ compared with control group, $^{\#}P < .05$ compared with pBoNT/C only, and $^{\xi}p < .05$, day 35 versus day 63. *Ruwona TB, Xu H, Li J, Diaz-Arévalo D, Kumar A, Zeng M, et al. Induction of protective neutralizing antibody responses against botulinum neurotoxin serotype C using plasmid carried by PLGA nanoparticles. Hum Vaccines Immunother 2016;12(5):1188–92 with permission.*

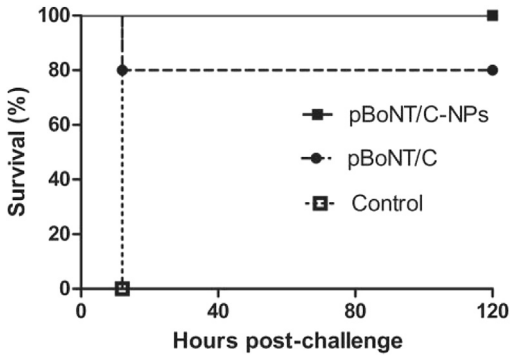

FIGURE 7.6 Protective immunity against BoNT/C challenge in immunized mice. SKH-1 Elite mice (n = 5) were dosed in weeks 0, 2, 4, and 8 with pVax/opt-BoNT/C-Hc50 plasmid (20 μg per mouse), plasmid alone (pBoNT/C), or plasmid coated onto PLGA NPs (pBoNT/C–NP), with control mice receiving PBS only and challenged in week 12 with 100 ± MLD50 of BoNT/C toxin. *Ruwona TB, Xu H, Li J, Diaz-Arévalo D, Kumar A, Zeng M, et al. Induction of protective neutralizing antibody responses against botulinum neurotoxin serotype C using plasmid carried by PLGA nanoparticles. Hum Vaccines Immunother 2016;12(5):1188–92 with permission.*

adult vaccination [44]. Furthermore, this company is working in recombinant trivalent nanoparticle influenza vaccine with matrix M-1. The vaccine can induce responses to one or more conserved HA head and stem epitopes. The antibody responses are able to neutralize against both homologous and heterologous strains [45].

The nanoparticles have been used in recent years in only a few of advanced clinical trials. The vaccine against RSV infection has three studies using RSV F nanoparticle vaccine with aluminum in phase 2 and 3 trials. The first clinical study of RSV was RSV F Dose-Ranging Study in Women, which started October 2013 and finished May 2016. The study showed that all formulations were well tolerated, without treatment-related serious adverse events. Antibodies anti-F IgG and palivizumab-competitive antibody responses were correlated and increased after both doses, while microneutralization assays increased significantly after the first dose, then plateaued [46]. The second study was RSV F Vaccine Maternal Immunization Study in Healthy Third-trimester Pregnant Women, which started September 2014 and finished June 2017. This study demonstrated that the vaccine is safe to infants and pregnant women. The third clinical study is in phase three trials and will finish in July 2019. The vaccine is safe and effective for infants and pregnant

women [44]. The study "Evaluation of the Safety and Immunogenicity of a Recombinant Trivalent Nanoparticle Influenza Vaccine with Matrix M-1 Adjuvant (NanoFlu)" was used to test protection in people older than age 60, but no results have been publicly released. The other study is the phase two trial entitled "Dose and Formulation Confirmation of Quad-NIV in Older Adults." In this study, 1375 subjects were randomized to seven treatment groups to receive the vaccine or an active comparator. Table 7.3 summarizes the clinical trials [47]: phase I–IV clinical trials/vaccines/nanoparticles.

1.9 Nanocytotoxicity

Engineered NPs have revolutionized the delivery of drugs, monoclonal antibodies, and vaccines to target cells. NPs have been designed from metals and nonmetals, polymeric materials, lipids, VLPs, and bioceramics, giving distinctive physicochemical and electrical characteristics that specifically interact with a targeted cell or organ. However, NPs can enter easily into the human body by crossing lipid bilayers and may interact with sensitive organs [48]. The clearance and excretion of NPs is mediated through the mononuclear phagocytic system, the renal urinary system, and by biliary clearance. Macrophages phagocytize the NPs and keep them in the secondary lymph organs and/or liver using the Mertk (Mer) receptor (the same receptor tyrosine kinase family as Axl and Tyro-3), which is responsible for promoting apoptotic cell engulfment and supports platelet aggregation and clot stability in vivo [49]. In addition, Kupffer cells, which are liver macrophages, sequester 100-nm nanoparticles [50]. When the particles are $\leq$5 nm in diameter, they are rapidly cleared from the circulatory system via renal filtration [51]. On the other hand, some studies have shown that metallic NPs (MeNPs) have effects on innate immunity. Specifically, in vitro assays showed that MeNPs have some cytotoxicity and genotoxicity and interfere with cytokine production and gene expression due to receptor modifications. The response of the innate immune system to threats is mediated by inflammation and the production of cytokines, chemokines, free radicals (nitric oxide) [52], and other reactive oxygen species

TABLE 7.3 Phase I and III clinical trials with vaccines delivery by nanoparticles.

Title	Infectious diseases	ClinicalTrials.gov identifier:	Phase
Evaluation of the safety and immunogenicity of a recombinant trivalent nanoparticle influenza vaccine with matrix M-1 adjuvant (NanoFlu)	Influenza	NCT03293498	I and II
Dose and formulation confirmation of Quad-NIV in older adults	Influenza	NCT03658629	II
RSV F dose–ranging study in women	Respiratory syncytial virus infections	NCT01960686	II
RSV F vaccine maternal immunization study in healthy third-trimester pregnant women.	Respiratory syncytial virus infections	NCT02247726	II
A study to determine the safety and efficacy of the RSV F vaccine to protect infants via maternal immunization	Respiratory syncytial virus infections	NCT02624947	III

(ROS). In this process, there is an accumulation of oxidized glutathione (GSSG), generating stress from the proinflammatory signal (involving TLRs) on the MeNPs and causing cell death and cancer [53]. Other studies have shown that adenovirus VLPs combine with a gene to control Gelsinger's ammonia metabolism (encoding ornithine transcarbamylase), which invades all the organs and induces severe reactions that can lead to death [54]. Other severe side reactions have been reported as carcinogenesis or germline alterations observed in animal models, in which the VLPs can become widely dispersed in the body, and the viral vector could end up in the ovaries and testes [55]. Eudragit is a drug delivery polymer: poly (ethyl acrylate-co-methyl methacrylate-cotrimethylammonioethyl methacrylate chloride) 1:2:0.1. It has been reported that using NR8383 macrophages cocultured with NP/ERS, the NPs were close to the inner membrane of the mitochondria, as observed by transmission electronic microscopy. The authors also analyzed genes responsible for mitochondrial function by microarray and found that *Opa1* was reduced in expression. This gene helps to maintain network morphology and dynamics and in the regulation of the signaling pathways for cell death, and NP/ERS-treated cells reduced glutathione (GSH), stimulating ROS production. Due to unbalancing of oxidant–antioxidant homeostasis and changes of proteins implied in activation and autophagy, the mitochondria decay through phagophore and autophagosome formation, and mitophagy can occur [11].

The NPs are characterized for their size (<100 nm) with greater surface area per mass compared with larger-sized particles of the same chemistry causing NPs more active biologically. The physicochemical properties of the material could increase the uptake by the antigen-presenting cells, but they can go to other tissues that can generate adverse biological effects such as apoptosis or necrosis. There are some materials that can be degraded easily. However, the NPs (PLGA) had been modified to release antigens slowly to immunize only once and to increase the efficiency of protective adaptive immune responses. Nevertheless, intranasal immunization with NPs had been reported to cause lung injury through oxidative stress, which induced the production of cytotoxic cellular responses and inflammatory cytokines. Another problem with the NPs is the aggregation that may block the blood vessels in the host. Modifications of polymeric NPs with PEI have been used to prevent the recognition to other cells such as epithelial cells in the lung. Other technologies could be used as the single-chain fragment region of antibodies binding to NPs, which recognizes the specific receptor on dendritic cells or macrophages that may deliver the antigens to target organ. Other modifications in the NPs have been used as PLGA covered with PEG (PLGA–PEG) and formulated with tetanus toxoid peptide–FITC linked to a Lys–Lys cathepsin cleavage site and an anti-DC-SIGN antibody, which help the delivery to DCs but not macrophages, with enhanced efficient proliferation of T cells [56]. Negatively charged NPs prevent the activation of the immune system and induction of immunotolerance, therefore preventing the effect of exacerbated inflammatory responses. Nanoparticles can also be engineered to be porous to increase the diffusion of intracellular proteases, resulting in earlier processing of APCs and presentation T cells [57]. In addition, some NPs carrying antigen and adjuvant in the same NP are less efficient to induce the immune response than the ones with adjuvant separated from antigens in different NPs. This may because of the competing effect of coactivation in several pathways [58].

2. Conclusion

NPs are widely explored in vaccine delivery and targeting APCs, especially DCs. Despite

advances in immunology, it is critical to understand the mechanisms of entry of NPs into cells and activation of the adaptive immune response and related toxicity. Some approaches inhibit the cytokine and chemokine cascade with DCs and T and B cells. NPs have great potential in immunology, although in-depth study of toxicity is necessary. In addition, NPs require molecules, such as antibodies or ligands, that bind to receptors on target cells to ensure that NPs only adhere to those cells. Any modification should be tested in cell lines or animals to prevent side effects or death. The synthesis of NPs can be shifted to methods that generate reproducible nanoparticles in terms of size (<10 nm, allowing elimination by the kidney), shape, and composition (biodegradable materials) to reduce cytotoxicity.

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